J I T A A
Journal of the Indonesian Tropical Animal Agriculture
Accredited by Ditjen Riset, Teknologi dan Pengabdian
kepada Masyarakat No. 164/E/KPT/2021

J. Indonesian Trop. Anim. Agric.
pISSN 2087-8273 eISSN 2460-6278
http://ejournal.undip.ac.id/index.php/jitaa
47(4):265-276, December 2022
DOI: 10.14710/jitaa.47.4.265-276

Supplementation of guanidinoacetic acid in feed with different levels of protein on intestinal histomorphology, serum biochemistry, and meat quality of broiler

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Received June 25, 2022; Accepted November11, 2022

ABSTRACT

This study aimed to investigate the effect of guanidinoacetic acid (GAA) with different protein levels in feed on intestinal histomorphology, serum biochemistry, and meat quality of broiler chicken. GAA is the only direct precursor of creatine and is involved in the synthesis of bioenergy cellular. This research used 1.176 day-old chicks of the Lohmann Indian River strain that were reared for 35 days. The supplementation effect was investigated using six treatments, i.e., two levels of feed protein and three levels of GAA, with seven replications. Broiler chickens were fed with 23% and 21% crude protein during the pre-starter phase, 21% and 19% during the starter phase, and 19% and 17% during the finisher phase, with a GAA addition of 0 g/ton, 600 g/ton, and 1200 g/ton, respectively. The result showed GAA supplementation with different protein levels reduced triglyceride ($P<0,05$), cholesterol levels in serum ($P<0,05$), increased surface area of the microvilli ($P<0,05$), pH of breast meat ($P<0,05$), but did not affect the chemical quality of breast meat ($P>0,05$). In conclusion, GAA supplementation in different level protein diets increased the surface area of the microvilli, pH of breast meat, reduce serum cholesterol and triglycerides, without affecting the chemical quality of broiler meat.

Keywords: broiler, GAA, intestinal histomorphology, meat quality

INTRODUCTION

The demand for broiler chicken in Indonesia has increased year after year, prompting the industry to develop and increase broiler chicken production. Currently, feed is a significant part of the production costs in the livestock business, necessitating a strategy to replace high protein feed ingredients with amino acids, which are considered more feasible in several ways. Poultry relies heavily on the supply of arginine because it is unable to synthesize *de novo* due to the lack of activity of the enzymes carbamoyl phosphate synthetase l, hepatic arginase, and ornithine carbamoyl transferase in the urea cycle (Abubados *et al*., 2014). Guanidinoacetic acid (GAA) is a component of compounds formed from arginine and glycine and this component is produced through the synthesis of glycine cyanamide, commercially known as CreAMINO. GAA also plays a vital part in muscle energy homeostasis (DeGroot *et al*., 2017). Furthermore, Dilger *et al*. (2013) reported that GAA can be used as an effective substitute for arginine in poultry feed.

Guanidinoacetic acid (GAA) is the only direct precursor in the formation of creatine in vertebrates (Lemme *et al*., 2007; Ostojic, 2014). This reaction occurs in the liver and tissues that require high energy, which then creatin will be carried to muscles and other target cells. After entering the cell, creatine is phosphorylated into phosphocreatine by creatine kinase and plays a role in buffering ATP changes. The addition of GAA has been thought to be unstable up to this time, requiring more research and investigations. Kharbanda *et al*. (2014) reported that the use of GAA in feed also has a negative side in such that it triggers several losses in metabolic responses (e.g., methylation of GAA to creatine using methyl), which may limit the availability of methyl for other important reactions, including DNA/ RNA methylation and the methionine synthase cycle.

In this study, the effect of GAA was thoroughly evaluated on how it is metabolized and its effect on meat products. The process of metabolism and digestion of nutrients affects the quality of meat (Gregory, 2010). The small intestine is the main organ for the process of digestion and absorption of digestive products in poultry. A healthy digestive tract of broiler chickens can be characterized by the development of weight and length of the digestive tract as well as the development of optimal intestinal villi to optimize nutrient absorption. Several studies have reported the positive effects of GAA supplementation on broiler feed, namely increasing productivity (Michiels *et al*., 2012; Mousavi *et al*., 2013; Fosoul *et al*., 2018), meat quality (Michiels *et al*., 2012). ; Liu *et al*., 2015), and small intestine health (Ahmadipour *et al*., 2018). However, studies regarding GAA supplementation in combination with different levels of protein have not been carried out. This study was conducted to determine the effect of GAA supplementation levels on feed with low and high protein content on growth performance, intestinal histomorphology, serum biochemistry, and meat quality of broiler chickens.

MATERIALS AND METHODS

Experimental Design and Diets

This experiment was designed as a 2 X 3 factorial with varying protein levels treatment and dietary GAA supplementation. A total of 1176 broiler chickens were divided to 6 treatment groups containing 7 replicate cages of 28 birds/cage with a ratio of 16 males and 12 females. This experiment used ration materials with varying protein levels and GAA concentration during three phases, i.e., 23% and 21% of crude protein (CP) in the pre-starter phase, 21% and 19% of CP during the starter phase, and 19% and 17% of CP during the finisher phase, with GAA addition of 0 g/ton, 600 g/ton, and 1200 g/ ton, respectively (Table 1). Broiler chickens were reared for 35 days without antibiotics and vitamins, comprising the pre-starter (0-11day), starter (12-21day), and finisher phase (22-35day). Maintenance was carried out in the Closed House of Universitas Gadjah Mada.

Growth Performance

Birds were weighed at 35 days of age and the feed consumption of birds for each replicate was recorded to determine final body weight, feed intake, and feed conversion ration (FCR).

Sampel Collection

At 35 days of age, blood samples were collected from the vena pectoralis into test tube, serum was separated by centrifugation at 3000 X g for 20 min at 4°C. The serum samples were frozen at -20° until further analysis. Samples for intestinal histomorphology test were randomly collected from three birds from each replicate. After slaughtering, 2 cm jejunum were immediately put into a 10% neutral buffer formalin fixative solution. Meat sampling for chemical and physical analysis of meat was carried out using 2 birds per replication. After deboning, the chicken

Table 1. Dietary composition and nutrients during growing period

Ingredients (%)	Prestarter		Starter		Finisher	
	High	Low	High	Low	High	Low
Maize	49.97	57.23	51.43	57.02	53.68	53.68
Soy Bean Meal	18.11	19.96	13.31	10.59	4.80	1.00
Corn Glutten Meal	2.50	1.50	1.62	1.00	1.60	0.50
Meat Bone Meal	1.50	1.00	2.10	2.29	3.84	3.89
Palm Kernel Meal	3.00	3.00	6.00	6.00	9.07	9.50
Full Fat Soya	18.80	10.56	19.22	15.50	20.19	19.77
Palm oil	1.99	1.97	2.59	2.50	3.65	3.65
L-Lysine	0.37	0.48	0.31	0.43	0.33	0.43
DL-Methionine	0.23	0.34	0.18	0.26	0.16	0.21
L-Threonine	0.12	0.23	0.07	0.16	0.07	0.13
Limestone	1.07	1.18	0.77	0.76	0.12	0.13
Biofos	0.30	0.51	0.07	0.07	0.01	0.01
NaCl	0.21	0.21	0.20	0.20	0.18	0.18
Sodium Bicarbonat	0.21	0.21	0.20	0.20	0.18	0.18
Avalia Se	0.02	0.02	0.02	0.02	0.02	0.02
Availa Mn	0.03	0.03	0.03	0.03	0.03	0.03
Availa Zn	0.03	0.03	0.03	0.03	0.03	0.03
Calculated nutrient level						
Dry matter (%)	88.46	88.29	88.54	88.46	88.54	88.65
Crude protein (%)	23.33	21.20	21.55	19.50	21.55	17.64
Crude fat (%)	7.15	6.14	8.07	7.63	8.07	9.66
Ash (%)	5.45	5.32	4.93	4.63	4.93	4.05
Starch (%)	33.36	37.38	33.96	37.23	33.96	36.85
ME (Kcal)	3.045	3.045	3.100	3.100	3.100	3.200
Ca (%)	0.96	0.96	0.87	0.87	0.87	0.78
Total P (%)	0.69	0.71	0.76	0.78	0.76	0.74
P available (%)	0.48	0.47	0.44	0.43	0.44	0.45
Methionine (%)	0.81	0.79	0.75	0.75	0.71	0.73
Met+Cys (%)	1.00	1.00	0.91	0.91	0.84	0.84
Lysine (%)	1.35	1.35	1.20	1.20	1.08	1.08

breasts and thighs were stored at -20°C for further analysis.

Intestinal Histomorphology

Tissue samples were analysed according (Dono, 2012). Samples were hydrated by passing a series of alcohols with different concentrations ranging from 70%, 80%, 90%, and 100%. The purification was carried out with a silol solution, then blocked in paraffin, then the tissue was cut with a 5μm thick microtome and placed on an object glass. The samples then observed and measured the villi height, villi width, crypt depth, and the ratio of villi height to crypt depth.

Serum Biochemistry

Serum biochemistry consists of levels of creatine, total cholesterol, triglycerides, albumin, urid acid, protein and glucose. Analysis of glucose levels using the GOD PAP photometric enzymatic method. Analysis of cholesterol using the CHOD PAP, photometric enzymatic method. Analysis of triglyceride using the GPO, photometric enzymatic method. Analysis of albumin using the photometric with bromocresol blue method. Analysis of protein levels using the Lowry method and analysis of uric acid levels using the Follin-Wu method.

Chemical Composition of Meat

The quality of meat using breast meat, the chemical composition of the meat tested included dry matter, ash, crude protein, crude fat and cholesterol levels. Crude protein, crude fat, ash and dry matter were analyzed using proximate analysis according to AOAC (2005). Determination of meat cholesterol using the Lieberman-Burchard method.

Physical Quality of Meat

The physical quality of meat tested included cooking loos and meat pH were tested according to the method of Bouton and Harris (1972), the water holding capacity was tested by Hamm's method, and tenderness was tested by shear press according to the Warner-Bratzler method.

Statistical Analysis

The data obtained from the study were analyzed using the analysis of variance following a 2 x 3 factorial, i.e., two levels of protein (high and low) and three levels of GAA (0 g/ton, 600 g/ton, and 1200 g/ton). If there is an indication of a significant difference, the data is further examined using the Duncan Multiple Range Test with the R program application.

RESULTS AND DISCUSSION

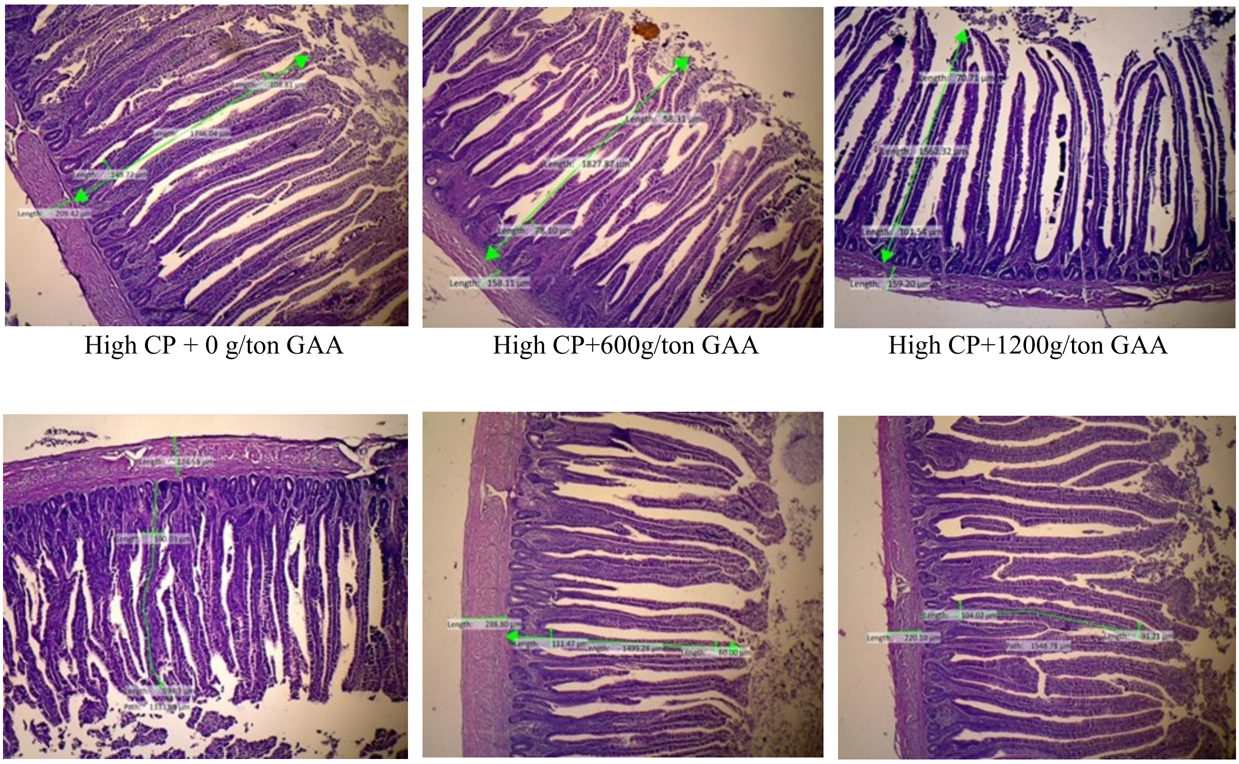
Growth Performance

Dietary supplementation of GAA did not show significant effect on on the cumulative feed intake, weight gain, FCR, performance index (IP) and mortality throughout the experimental period (data not shown). However, high protein and low protein content on feed showed significant effect on cumulative feed intake (2.166,16 g vs 2.030,30 g), weight gain (1.250,89 g vs 1.035,17 g), FCR (1,64 vs 1,83), and IP (152,85 vs 109,10) ($P<0,05$). Our result is supported by the finding of El-Faham (2019) who found that supplementation up to 0,12% of GAA did not affect feed intake. Nasiroleslami *et al*. (2018) also found that dietary 0,12% GAA did not show significant effect on feed intake, body weight gain, and FCR. Other results reported supplementation of GAA significantly improve FCR (Mousavi *et al*., 2013). GAA supplementation might be important in poultry nutrition to support energy homeostasis of chicken broiler, especially in chickens broiler fed the creatine-deficient diets exclusively based on vegetable ingredients (Michiels *et al*., 2012; Dilger *et al*., 2013).

Intestinal Histomorphology of Broiler Chicken

The histomorphology of broiler chicken intestines that were fed with the supplementation of guanidino acetic acid with different protein levels was examined through the jejunum observation, i.e., villi height and width, crypt depth, and surface area. The results show that the interaction of GAA with different protein levels is not significantly ($P>0.05$) affect villi height and crypt depth, but it significantly influences the surface area and width of the villi ($P<0.05$). Meanwhile, differences in protein levels showed significant results ($P<0.05$) on villi height and surface area. Moreover, the addition of GAA showed significant results ($P<0.05$) on crypt depth (Figure 1).

Villi height is closely related to nutrient absorption (Mantis *et al*., 2011), whereas crypt depth is considered as villous factory and crypts show rapid tissue turnover and high demand for new tissue (Ndazigaruye *et al*., 2019). Protein and amino acid balance play a major role in gut health and performance (Barekatain *et al*., 2019). This study shows that the addition of 0.06% GAA increases the crypt depth, which normally indicates a fast and high network demand for



High CP + 0 g/ton GAA | High CP+600g/ton GAA | High CP+1200g/ton GAA

Low CP + 0 g/ton GAA | Low CP+600g/ton GAA | Low CP+1200g/ton GAA

Figure 1. Intestine Histomorphology of Broiler Chicken

new networks. However, the addition of GAA at the level of 0.12% decreased the crypt depth. This result shows that GAA can act as a alternative for arginine (Baker, 2009) which can stimulate cell migration in the intestine through the NO and focal adhesion kinase pathway (Rhoads *et al*., 2004). Arginine plays an important role in the synthesis of polyamines which participate in cell division (Tan *et al*., 2010).

Protein and amino acid balance play a major role in gut health and performance (Barekatain *et al*., 2019). Villi height is closely related to nutrient absorption (Mantis *et al*., 2011). Although high crude protein can increase villi height, it is thought to be related to nutrient absorption by broiler chickens, which is followed by an increase in surface area. The addition of 0.12% GAA in high crude protein shows the maximum villi width. An increase in the width of the small intestinal villi could optimize the surface absorption, and this is presumably due to feeding conversion in the addition of 0.12% GAA with high crude protein.

Serum Biochemistry of Broiler Chicken

The serum biochemistry of broiler chickens administered with GAA with different protein levels in the feed is shown in Table 3. The analysis shows that the interaction of GAA with different protein levels has a significant effect ($P<0.05$) on total cholesterol and triglycerides. The addition of GAA can reduce blood cholesterol levels when it combined with low crude protein treatment, while the addition of GAA can reduce triglyceride levels in high crude protein treatment. The level of triglycerides in this study ranged from 61.34 to 112.55 mg/dL. As a comparison, Boroumandita *et al*. (2021) reported that the range of triglyceride levels in the addition of 3% GAA is ranged from 47.14 to 52.73 mg/dL. Our results show that GAA supplementation in high-protein or low-protein diets can reduce triglyceride or serum cholesterol levels. Blood cholesterol or triglyceride levels can be related to blood creatinine levels. In this study, the interaction between GAA and feed protein levels did not have a significant effect on blood creatinine levels (P>0.05). In the high protein diet, the addition of GAA decreased creatinine levels, although not significantly. Creatinine can affect blood triglyceride and cholesterol levels through the activation mechanism of a protein that plays a role in fat metabolism (apolipoprotein A) and 14 kDa Apo-A which is involved in cholesterol transport and lipid metabolism (Geay *et al*., 2011; Moreira *et al*., 2017). Decreased blood creatinine levels have the potential to reduce cholesterol and lipid levels in the blood.

The addition of GAA with different protein levels in the feed showed non-significant results (P>0.05) on the total protein, which is ranging from 2.18-3.07 g/dL. The total protein includes all types of protein found in the blood, such as albumin, globulin, and several other proteins. Total protein content was influenced by feed consumption and correlated with albumin levels. In this study, the interaction between GAA supplementation and protein level has no significant effect (P>0.05). The total protein is influenced by nutritional status and feed intake as well as the effectiveness of metabolic processes. GAA is a precursor for the formation of creatine and can act as an alternative to arginine. Supplementation of GAA will increase the availability of arginine for protein accretion because the arginine required for the formation of creatine can be met through GAA (Portocarero and Braum, 2021). In this study, supplementation of GAA has no significant effect on serum protein which could be related to feeding consumption. In this study, more GAA supplementation caused a decrease in feed consumption, although not significantly. Lower feed consumption causes a lower intake of protein needed by the body, which has implications for blood protein levels.

Glucose is an organic compound that functions as an energy source. Blood glucose content is closely related to carbohydrate intake from feed. Supplementation GAA in high-protein and low-protein diets has no significant effect on blood glucose levels (P>0.05). Our finding shows that GAA supplementation did not affect blood glucose availability, although GAA supplementation caused a decrease in feed con-

Table 2. Intestine Histomorphology of Broiler Chicken

Parameter	GAA (g/ton)	Crude Protein		Average
		High	Low	
Villus height (mm)	0	1.80±0.13	1.22±0.32	1.51±0.38
	600	1.83±0.22	1.39±0.07	1.61±0.28
	1200	1.42±0.20	1.38±0.25	1.40±0.21
Average		1.69±0.26[a]	1.33±0.23[b]	
Villus width (mm)	0	0.12±0.04[ab]	0.10±0.02 [ab]	0.11±0.03
	600	0.09±0.01 [b]	0.12±0.02 [ab]	0.10±0.02
	1200	0.14±0.02 [a]	0.10±0.01 [ab]	0.12±0.02
Average		0.11±0.02	0.10±0.02	
Crypt depth (mm)	0	0.20±0.01	0.21±0.02	0.21±0.01[b]
	600	0.28±0.09	0.31±0.05	0.30±0.07[a]
	1200	0.22±0.04	0.22±0.04	0.22±0.03[b]
Average		0.24±0.06	0.26±0.05	
Surface area (mm)	0	0.68±0.21[a]	0.36±0.03[b]	0.52±0.22
	600	0.51±0.09[ab]	0.53±0.09[ab]	0.52±0.08
	1200	0.63±0.12[a]	0.44±0.05[b]	0.54±0.13
Average		0.61±0.16[a]	0.44±0.09[b]	

[a,b] different superscripts in the same column/row is significantly different (P<0.05)

Table 3. Serum Biochemistry of Broiler Chicken

Parameter	GAA (g/ton)	Crude Protein		Average
		High	Low	
Creatin (mg/dL)	0	0.14±0.01	0.10±0.01	0.12±0.02
	600	0.13±0.02	0.12±0.02	0.13±0.02
	1200	0.12±0.03	0.12±0.02	0.12±0.02
Average		0.13±0.02[a]	0.11±0.02[b]	
Albumin (g/dL)	0	1.01±0.10	1.42±0.07	1.21±0.23
	600	1.19±0.17	1.32±0.25	1.25±0.20
	1200	1.17±0.15	1.24±0.19	1.21±0.16
Average		1.12±0.15[b]	1.33±0.18[a]	
Glucose (mg/dL)	0	4.93±0.95	9.43±5.47	7.18±4.29
	600	6.36±4.35	5.33±1.42	5.85±2.95
	1200	4.83±1.90	3.66±1.24	4.25±1.57
Average		5.37±2.53	6.14±3.87	
Cholesterol (mg/dl)	0	94.90±6.33[b]	172.53±13.06[a]	133.71±43.50[a]
	600	101.53±15.00[b]	121.36±31.70[b]	111.45±24.70[b]
	1200	94.03±5.83[b]	97.26±15.05[b]	95.65±10.36[b]
Average		96.82±9.34[b]	130.38±38.19[a]	
Triglyceride (mg/dL)	0	112.55±8.52[a]	61.95±4.21[b]	87.25±28.35[a]
	600	79.14±20.71[b]	66.23±9.78[b]	72.68±16.11[b]
	1200	63.44±9.81[b]	61.34±3.80[b]	62.39±6.75[b]
Average		85.04±24.92[a]	63.17±6.10[b]	
Protein (g/dL)	0	2.62±0.02	2.32±0.35	2.47±0.27
	600	2.90±0.44	3.07±0.75	2.98±0.56
	1200	2.69±0.54	2.18±0.60	2.43±0.58
Average		2.73±0.37	2.52±0.66	
Calcium (mg/dL)	0	10.72±1.62	9.70±1.85	10.21±1.66
	600	9.82±0.31	12.47±1.44	11.14±1.72
	1200	10.01±2.03	13.63±1.54	11.82±2.56
Average		10.19±1.37[b]	11.93±2.24[a]	
Uric acid (mg/dL)	0	1.03±1.07	2.26±1.61	1.65±1.40
	600	1.86±0.89	3.03±2.53	2.44±1.81
	1200	2.55±2.59	1.29±1.15	1.92±1.72
Average		1.81±1.61	2.19±1.77	

[a,b] different superscripts in the same column/row is significantly different ($P<0.05$)

sumption. This result could be due to the presence of GAA supplementation in low-protein diets that increased the surface area of the villi so that nutrient absorption could be more optimal (Table 2). Our finding also shows that in meeting the requirements of glucose, the body does not use protein or amino acids as indicated by uric acid levels that are no different. Our result is in line with Fosoul *et al*. (2018) that stated GAA supplementation increased protein and energy use.

Uric acid is a by-product of nitrogen and protein metabolism (Sulistyoningsih *et al*., 2015). Blood uric acid level is likely associated with protein utilization as an energy source when birds encounter energy restriction (Fosoul *et al*., 2018). Our finding shows that supplementation GAA with different protein levels showed insignificant results (P>0.05) on blood serum uric acid levels in broiler chickens. The results of this study indicate that a decrease in the protein content of feed and GAA supplementation did not interfere with normal protein metabolism. This result is related to uric acid levels in low protein diets, which decreased with the addition of GAA supplementation. This result indicates that the presence of GAA supplementation can increase the efficiency of energy and protein use in feed. This result is in line with Fosoul *et al*. (2018), who reported that GAA supplementation increased protein and energy use.

GAA supplementation with different protein levels showed insignificant results (P>0.05) on blood calcium levels. The addition of GAA had no significant effect (P>0.05), while different protein levels had a significant effect on the calcium content in blood serum. Serum calcium levels were higher in the low protein diet than in the standard protein. Boroumandnia *et al*. (2021), reported that GAA supplementation of up to 3% resulted in calcium levels of 7.79-8.58 mg/dL. In this study, GAA supplementation with different protein levels resulted in blood serum calcium levels ranging from 9.70-13.63 mg/dL, which were higher than Boroumandnia *et al*. (2021). This study showed that high-protein feed treatment led to higher blood calcium levels. It is apparently because the height of the villi and the

Table 4. Chemical Quality of Broiler Chicken Meat

Parameter	Level of GAA (g/ton)	Crude Protein (%)		Average
		High	Low	
Dry Matter (%)	0	26.41±1.92	24.89±0.80	25.65±1.60
	600	25.61±1.10	25.14±0.72	25.37±0.91
	1200	25.21±0.53	24.80±0.72	25.00±0.63
		24.94±0.70	25.74±1.32	
Ash (%)	0	1.21±0.15	1.32±0.10	1.27±0.14
	600	1.38±0.30	1.46±0.22	1.42±0.25
	1200	1.34±0.11	1.43±0.17	1.39±0.14
		1.31±0.21	1.40±0.17	
Crude Protein (%)	0	23.63±0.84	22.76±1.86	23.19±1.44
	600	22.02±0.69	22.35±1.09	22.18±0.88
	1200	22.33±1.75	23.75±1.85	23.04±1.86
		22.66±1.32	22.95±1.64	
Crude Fat (%)	0	3.91±0.39	3.13±0.71	3.52±0.68
	600	3.12±1.20	2.59±0.46	2.85±0.90
	1200	3.19±0.79	2.83±0.72	3.01±0.74
		3.41±0.87	2.85±0.63	
Cholesterol (mg/100g)	0	40.22±3.09	44.00±5.11	42.11±4.46
	600	40.49±2.68	43.37±5.19	41.94±4.18
	1200	43.62±2.74	42.09±5.17	42.86±3.98
		41.44±3.08	43.15±4.84	

[a,b] different superscripts in the same column/row is significantly different (P<0.05)

Table 5. Physical Quality of Broiler Chicken Meat

Parameter	Level of GAA (g/ton)	Level of Protein (%)		Rata-rata
		Tinggi	Rendah	
pH	0	5.79±0.07[b]	5.97±0.12[a]	5.88±0.13
	600	5.94±0.18[ab]	5.84±0.07[ab]	5.89±0.14
	1200	5.87±0.12[ab]	5.73±5.56[ab]	5.88±0.10
		5.87±0.14	5.90±0.10	
WHC (%)	0	51.86±11.39	57.17±5.27	54.52±8.82
	600	55.75±6.15	55.25±6.24	55.50±5.84
	1200	52.70±6.06	58.73±5.56	55.71±6.34
		57.83±7.83	57.05±5.48	
Tenderness (kg/cm^2)	0	1.74±0.50	2.34±0.71	2.04±0.66
	600	2.16±0.74	1.79±0.27	1.97±0.56
	1200	2.55±0.77	1.90±0.67	2.22±0.76
		2.15±0.72	2.01±0.60	
Cooking Loss (%)	0	29.86±4.26	32.37±4.58	31.12±4.38
	600	29.05±3.72	26.75±1.17	27.90±2.87
	1200	30.75±2.11	27.69±1.73	29.22±2.43
		29.89±3.30	28.94±3.70	

[a,b] different superscripts in the same column/row is significantly different (P<0.05)

larger surface area of the villi in the high protein diet resulted in better absorption of the nutrient.

Chemical Quality of Broiler Chicken Meat

Table 4 shows the chemical quality of broiler meat given GAA with different protein levels in the feed. Based on the analysis, the addition of GAA with different protein levels was not significant (P>0.05) on dry matter, ash, crude protein, crude fat, and cholesterol. The chemical quality of meat is closely related to the nutritional content of the meat itself, while the nutritional content is related to the quality of the food itself. Lawrie (1995) reported that age, race, species, stress, feed, and gender are affecting the chemical quality of meat. In this study, the addition of GAA was able to reduce blood cholesterol levels at low crude protein treatment (Table 3) but was not able to reduce cholesterol levels in meat. Cholesterol levels in this study ranged from 40.22 to 43.62 mg/100g, which is lower than normal cholesterol levels (56 mg/100mg) as reported by Ponte *et al*. (2008). The average protein content in this study is ranging from 22.02 to 23.63%, slightly higher compared to normal levels of 16-22% (Aberle *et al*., 2001). Liu *et al*. (2015) stated that the protein content of broiler meat is influenced by feed consumption because the amount of feed consumed determines the amount of protein deposited in the meat.

Physical Quality of Broiler Chicken Meat

Table 5 shows the physical quality of broiler meat given GAA with different protein levels in the feed. The analysis shows that the interaction of GAA with protein levels was significant to the pH value of meat; when GAA was added to feed with low crude protein, the meat has a higher pH value. The average pH value of meat in this study is 5.73 to 5.97. The result is supported by the findings of Liu *et al*. (2015), who reported that 0.1% GAA supplementation increased the pH of meat compared to control. Huff-Lonergan and Lonergan (2005) stated that GAA supplementation in feed was able increase the availability of energy sources such as phosphocreatine and ATP, which inhibited the conversion of glycogen to lactic acid, thereby maintaining postmortem pH. Inhibition of the decrease in pH value of postmortem meat has positive effect on water holding capacity (WHC) (Michiels *et al*., 2012).

GAA supplementation did not show significant effect on WHC, tenderness, and cooking loss. The cooking loss value in this study is ranging from 26.75 to 32.37%. The cooking loss value of meat in this study is still in the normal range. High meat pH also reduces cooking loss because it can inhibit protein denaturation when the meat temperature is still high (Huff-Lonergan and Lonergan, 2005). The average tenderness value in this study ranged from 1.74-2.55 kg/cm2. Cordova *et al*., (2018) reported that the tenderness value of meat with the addition of 600 g/ton GAA showed insignificant results with a tenderness value of 1.24 kg/cm^2.

CONCLUSION

The supplementation of GAA up to 0.12% in the feed can reduce triglycerides and cholesterol in broilers blood serum, especially when it combined with high crude protein levels, but this treatment unable to reduce cholesterol in broiler breast meat. The addition of 0.06% GAA is the optimal level for increasing the crypt depth of the small intestine and will decrease the surface area when it mixed with low the crude protein level. Overall, broilers that fed with the supplementation of GAA with low crude protein has a higher meat's pH value.

ACKNOWLEDGMENTS

This research project is a collaboration with Widodo Makmur Unggas and is funded by Universitas Gadjah Mada through the Batch I of *Rekognisi Tugas Akhir* (RTA) Program 2022.

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